The Tn*3* β-Lactamase Gene Acts as a Hotspot for Meiotic Recombination in Yeast

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ABSTRACT

Although genetic distances are often assumed to be proportional to physical distances, chromosomal regions with unusually high (hotspots) or low (coldspots) levels of meiotic recombination have been described in a number of genetic systems. In general, the DNA sequences responsible for these effects have not been determined. We report that the 5′ region of the β-lactamase (amp^R) gene of the bacterial transposon Tn*3* is a hotspot for meiotic recombination when inserted into the chromosomes of the yeast *Saccharomyces cerevisiae*. When these sequences are homozygous, both crossing over and gene conversion are locally stimulated. The 5′ end of the β-lactamase gene is about 100-fold "hotter" for crossovers than an average yeast DNA sequence.

IN yeast and other fungi in which all four products of meiosis can be recovered, two types of meiotic recombination are observed, crossing over and gene conversion. If a diploid is constructed that is heterozygous for two linked loci (one homolog containing the wild-type alleles *A* and *B*, and the other containing the mutant loci, *a* and *b*), tetrads with single crossovers have two spores with the parental combination of markers *AB* and *ab*, and two spores with the reciprocal products of recombination, *Ab* and *aB*. In these tetrads, each heterozygous marker segregates 2:2. Departures from 2:2 segregation in which tetrads contain either an extra wild-type spore (for example, 3*B*:1*b*) or an extra mutant spore (1*B*:3*b*) are called "gene conversion" events. Since meiotic gene conversion events are frequently associated with crossing over of flanking DNA sequences (FOGEL and HURST 1969), these two different types of recombination are assumed to be related mechanistically (HOLLIDAY 1964; MESELSON and RADDING 1975; RADDING 1982; SZOSTAK *et al.* 1983).

In *Saccharomyces cerevisiae*, there are about 0.37 cM/kb (STRATHERN *et al.* 1979), but a number of chromosomal regions that have unusually high or low levels of recombination have been observed. For example, the *CDC24-PYK1* interval of chromosome *I* has about 5-fold more crossovers than an average interval (COLEMAN *et al.* 1986) and the *LEU2-HIS4* interval of chromosome *III* is about 3-fold "hotter" than the average (NEWLON *et al.* 1986). A 2.9-kb region of chromosome *III* (between the G3 and B sites in Figure 1) was identified as a hotspot for crossovers (SYMINGTON and PETES 1988); recent evidence indicates that this region is a preferred site for the resolution of recombination events initiated in the B-M region of chromosome *III* (Figure 1; L. SYMINGTON and T. PETES, unpublished data). In addition, an insertion of the *LEU2* gene in an ectopic location on chromosome *III* was a hotspot for meiotic crossovers (CAO, ALANI and KLECKNER 1990).

The DNA sequences responsible for stimulating meiotic crossovers have not been determined with a fine degree of resolution in *S. cerevisiae*. In contrast, the M26 mutation in *Schizosaccharomyces pombe*, which results in a stimulation of both crossing over and gene conversion (GUTZ 1971; PONTICELLI, SENA and SMITH 1988; SCHUCHERT and KOHLI 1988; SZANKASI *et al.* 1988), is a single-base pair change within the coding sequence of the *ade6* gene; when a 3-kb DNA fragment containing the M26 mutation is moved to a different chromosomal location, no stimulation of meiotic recombination is observed (A. PONTICELLI and G. R. SMITH, personal communication).

The rate of meiotic gene conversion in *S. cerevisiae* varies about 40-fold between different loci and, in addition, often varies at different positions within a single gene (summarized by FOGEL, MORTIMER and LUSNAK 1981). In some yeast genes, an approximately linear gradient (a polarity gradient) of gene conversion frequencies is observed. The high-conversion end of the gene is the 5′ end for *ARG4* (FOGEL, MORTIMER and LUSNAK 1981; NICOLAS *et al.* 1989) and *HIS4* (M. WHITE, P. DETLOFF, M. STRAND and T. PETES, unpublished data), but the 3′ end for *HIS2* (R. MALONE, personal communication). For the *ARG4* gene, deletion of the promoter sequences reduced the rate of gene conversion (NICOLAS *et al.* 1989), indicating the possibility of an association between the initiation of

meiotic recombination and transcription; such an association has been observed for mitotic recombination in yeast (KEIL and ROEDER 1984; THOMAS and ROTHSTEIN 1989), although the reason for this effect is not yet understood. The conclusion that meiotic recombination is associated with transcription is complicated by two observations. First, not all deletions that remove the promoter of *ARG4* affect the rate of gene conversion (NICOLAS *et al.* 1989). Second, deletion of the promoter of *HIS4* has no significant effect on gene conversion at this locus (M. WHITE, P. DETLOFF, M. STRAND and T. PETES, unpublished data). In two studies, double-strand breaks have been detected within the hotspot DNA sequences (SUN, TRECO and SZOSTAK 1989; CAO, ALANI and KLECKNER 1990). No double-strand breaks have been detected thus far at the *HIS4* (M. WHITE, P. DETLOFF, M. STRAND and T. PETES, unpublished data) or *HIS2* (R. MALONE, personal communication) hotspots.

SEIFERT *et al.* (1986) have developed a system for mutagenesis of yeast chromosomes that is based on the bacterial transposon Tn*3*. As described below, we found that the β-lactamase gene derived from this transposon, when inserted homozygously into yeast chromosome *III*, strongly stimulates meiotic gene conversion and crossing over.

MATERIALS AND METHODS

Media and growth conditions: *Escherichia coli* were grown in LB medium (MANIATIS, FRITSCH and SAMBROOK 1982) at 37°. Cultures were supplemented with antibiotics [ampicillin (50 μg/ml), kanamycin (40 μg/ml), tetracyline (15 μg/ml)] when necessary.

Yeast cells were grown vegetatively at 32° in YPD medium (1% yeast extract, 2% peptone, 2% dextrose; SHERMAN, FINK and HICKS 1983). Strains were prepared for sporulation by inoculating 20 μl of a stationary phase culture into 5 ml of YPA (1% yeast extract, 2% peptone, 2% potassium acetate). These cultures were incubated with vigorous aeration for 16–24 hr. The cultures were then harvested by centrifugation and resuspended in 2.5 ml of 2% potassium acetate (supplemented with adenine to a concentration of 20 μg/ml). The cells were then incubated with vigorous aeration at 25° for 3 days. The sporulated cultures were dissected and the segregation of nutritional markers was analyzed by standard techniques (SHERMAN, FINK and HICKS 1983). Tetrads with a crossover between *LEU2* and *CEN3* were identified as tetrads that had tetratype segregation for *LEU2* and *TRP1* (SYMINGTON and PETES 1988); the crossover between *LEU2* and *CEN3* was confirmed by restriction analysis as described below. Strains that were mutant at the *ura3* locus were selected using 5-fluoro-orotic acid (5-FOA) as described by BOEKE, LACROUTE and FINK (1984).

Plasmid DNA isolation and manipulation: A description of the plasmids used in our study is given in Table 1. Most of these plasmids were derivatives of the plasmids A2C, C2G, D8B and G4B [constructed by NEWLON *et al.* (1986)] that contained *Bam*HI fragments derived from yeast chromosome *III* inserted into YIp5.

To isolate small amounts of plasmids, we used the procedure described by VOGELSTEIN and GILLESPIE (1979). For larger amounts (greater than 10 μg of DNA), the alkaline lysis method was used (MANIATIS, FRITSCH and SAMBROOK 1982). Digestions with restriction enzymes, purification of DNA fragments from low melting point agarose and DNA ligations were performed as described by MANIATIS, FRITSCH and SAMBROOK (1982). In some experiments, in order to create a heterozygous marker, the 3′ recessed ends resulting from restriction digestion of plasmid DNA were "filled-in" using DNA polymerase (SYMINGTON and PETES 1988). Bacterial transformations were done by standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982).

We selected insertions of the bacterial transposon mini-Tn*3*-*URA3* into yeast DNA sequences using the protocol of SEIFERT *et al.* (1986). *Bam*HI fragments of yeast DNA were cloned into the *Bam*HI site of the plasmid pHSS6 (Table 1). These recombinant plasmids were cotransformed with the plasmid pLB101 into the bacterial strain DH1 (*recA1 endA1 gyrA96 thi1 hsdR17 supE44*); pLB101 encodes the Tn*3* transposase but does not have a transposon. The resulting transformants were mated to a strain containing the F-factor derivative pOX38::mini-Tn*3*::*URA3* and bacterial strains containing all three plasmids were selected. The cells were grown at 30° to allow transposition of the mini-Tn*3* element into the recombinant plasmid containing the yeast sequences, resulting in formation of a cointegrate. Since the mini-Tn*3* element contained a site (*loxP*) derived from bacteriophage P1, this cointegrate was resolved by mating the strain with the plasmid cointegrate to the strain NS2114Sm which expresses a resolvase (*CRE*) that cleaves the *loxP* sites. The net result of these manipulations is a collection of plasmids with mini-Tn*3* inserted into the yeast and vector DNA sequences. We examined the position of the insertions in individual plasmids by restriction analysis.

Yeast strains and strain construction: A description of the yeast strains used in our study is given in Table 2. In general, these strains are isogenic (except for changes introduced by transformation) with the previously described strains LS42, LS45 and LS47 (SYMINGTON and PETES 1988). Each strain is heterozygous for multiple markers on chromosome *III*, resulting from "filling-in" restriction sites. In addition, most of the strains contain either heterozygous or homozygous insertions of mini-Tn*3*::*URA3*. Strains with these insertions were made by either one-step (ROTHSTEIN 1983) or two-step (SCHERER and DAVIS 1979; WINSTON, CHUMLEY and FINK 1983) transplacement procedures using the *URA3* gene in the transposable elements as the selective marker.

Analysis of segregation of heterozygous restriction site markers: In order to examine the segregation of the heterozygous restriction sites in tetrads with a crossover between *LEU2* and *CEN3*, we isolated DNA from 5 ml YPD cultures (SHERMAN, HICKS and FINK 1983) derived from the individual spores of the tetrad. To examine heterozygous sites in the C2G and D8B *Bam*HI fragments (Figure 1), we digested the DNA with either *Bgl*II, *Mlu*I, *Bcl*I, *Bam*HI or *Xho*I, separated the fragments by agarose gel electrophoresis, and transferred the fragments to HY-bond N (Amersham). The filter was hybridized to ^{32}P-labeled pLS47 [labeled by nick translation (MANIATIS, FRITSCH and SAMBROOK 1982) or random priming (FEINBERG and VOGELSTEIN 1983)] using the conditions recommended by the manufacturer. The pattern of hybridization was detected by autoradiography. Heterozygous sites near *CEN3* and *LEU2* were examined by using hybridization probes derived from the plasmids pAS16 and p7/313 (Figure 1).

Physical analysis of recombination: The methods that we used were similar to those described by BORTS *et al.* (1984). DNA was isolated from 3-day-old sporulated cul-

TABLE 1

Description of recombinant plasmids

Name	Description and source of plasmids
D8B	*Bam*HI fragment D (see Figure 1) from chromosome *III* cloned into YIp5, (NEWLON *et al.* 1986)
C2G	*Bam*HI fragment C (see Figure 1) from chromosome *III* cloned into YIp5 (NEWLON *et al.* 1986)
A2C	*Bam*HI fragment contiguous with C (see Figure 1) cloned into YIp5, contains DNA to right of *CEN3* (NEWLON *et al.* 1986)
G4B	*Bam*HI fragment G of chromosome *III* cloned into YIp5 (NEWLON *et al.* 1986)
C1G	*Bam*HI fragment from chromosome *III* that includes the *HIS4* gene cloned into YIp5 (NEWLON *et al.* 1986)
pLS32	G4B with 3 filled-in restriction sites,[a] *Bst*EII$^-$(*Mae*II$^+$), *Xho*I$^-$(*Pvu*I$^+$), *Bst*EII$^-$(*Mae*II$^+$); one of the fill-ins creates a *leu2* mutation (SYMINGTON and PETES 1988)
pLS36	Derived from insertion of a *URA3* gene into the *Bcl*I site of pLS32 (SYMINGTON and PETES 1988)
pLS47	D *Bam*HI fragment with restriction site fill-ins fused to C fragment with restriction site fill-ins in YIp5, *Bam*HI site between D and C also filled in (SYMINGTON and PETES 1988). The altered sites are: *Spe*I$^-$(*Alu*I$^+$), *Bgl*II$^-$(*Cla*I$^+$), *Xho*I$^-$(*Pvu*I$^+$), *Bgl*II$^-$(*Cla*I$^+$), *Bgl*II$^-$(*Cla*I$^+$), *Bam*HI$^-$(*Cla*I$^+$), *Bcl*I$^-$(*Cla*I$^+$), *Bgl*II$^-$(*Cla*I$^+$), and *Mlu*I$^-$(*Bss*HII$^+$).
pLS55	*Cla*I$^-$(*Nru*I$^+$) derivative of A2C (SYMINGTON and PETES 1988)
pLS57	*Eco*RI$^-$(*Xmn*I$^+$) derivative of C2G (SYMINGTON and PETES 1988)
pLS65	*Sal*$^-$(*Pvu*I$^+$) derivative of C1G (23); *his4* mutation caused by fill-in of *Sal*I (provided by L. SYMINGTON)
pRB35	YIp5 derivative (BORTS and HABER 1987), containing mutant *ura3* caused by filling-in of *Nco*I site to create *Nsi*I site.
pSH2::U3	Plasmid with *CAN1* gene and *URA3* gene in pBR322 (provided by S. LIEBMAN)
pHSS6	Plasmid vector used for cloning *Bam*HI fragments of yeast DNA in preparation for transposon mutagenesis (SEIFERT *et al.*, 1986). Contains *ori*, gene encoding kanamycin resistance, and a polylinker
p7/313	Plasmid containing *Eco*RI fragments derived from chromosome *III* near *CEN3* (see Figure 1); used as a hybridization probe (GAUDET and FITZGERALD-HAYES 1987)
pAS14	*Bam*HI fragment from D8B inserted in *Bam*HI site of pHSS6
pAS15	*Bam*HI fragment from G4B inserted in *Bam*HI site of pHSS6
pAS16	Derivative of pAS15 that has a deletion of Ty1 from pAS15 (constructed by L. SYMINGTON); used as a hybridization probe (see Figure 1)
pAS17	*Bam*HI fragment from C2G inserted in *Bam*HI site of pHSS6
pAS55	Plasmid with mini-Tn*3*::*URA3* transposed into pAS17 (site 1 insert, see Figure 1)
pAS56	Plasmid with mini-Tn*3*::*URA3* transposed into pAS14 (site 2 insert, see Figure 1)
pAS26	Derivative of pAS56 resulting from partial digestion with *Hin*dIII, followed by intramolecular ligation; deleted for 1.1-kb *Hin*dIII *URA3* fragment
pAS30	Plasmid constructed with *Bg*III fragment of pAS56 cloned into *Bam*HI site of pHSS6; includes mini-Tn*3*::*URA3* and flanking yeast sequences; substrate for preparing deletion of *amp*^{R} sequences from mini-Tn*3*
pAS31	Plasmid constructed by partial digestion of pAS30 with *Eco*RI, followed by intramolecular ligation; results in deletion of the *Eco*RI fragment that contains *amp*^{R}
pAS35	Plasmid constructed by inserting *Xba*I fragment derived from pAS14 in *Xba*I site of pHSS6; used as a hybridization probe (see Figure 3)
pAS57	Plasmid with insertion of mini-Tn*3*::*URA3* in pAS14 (site 3 insert; Figure 1)
pAS58	Plasmid with *Xho*I linker in *Sma*I site of pHSS6
pAS98	*Xba*I-*Xho*I fragment of pAS56 (contains site 2 insert) cloned into *Xba*I-*Xho*I digested pAS58; substrate for preparing deletions of site 2 mini-Tn*3* insertion
pAS101	Deletion derivative of pAS98; pAS98 was treated with *Kpn*I and *Ase*I, followed by *Exo*III digestion; *Exo*III removes nucleotides from one DNA strand from the end produced by *Ase*I, but not from the end produced by *Kpn*I. The resulting product is treated with mung bean nuclease (to remove the single-stranded DNA tails) and the ends are ligated together (HENIKOFF 1984). The resulting deletion is shown in Figure 5 (3′ *amp* deletion)
pAS103	Deletion derivative of pAS98; pAS98 was partially digested with *Hin*dIII and digested to completion with *Asp*718 (an isoschizomer of *Kpn*I that leaves a 5′ protruding end). The cohesive ends were filled-in with DNA polymerase and ligated together. The resulting deletion (*loxP amp*) is shown in Figure 5
pAS105	Deletion derivative of pAS101 involving a *Hin*dIII partial digest, followed by intramolecular ligation of the resulting fragment. The resulting deletion (*URA3* 3′*amp*) is shown in Figure 5
pAS106	Deletion derivative of pAS101. The plasmid was treated with digested with *Nsi*I to completion and the cohesive ends were filled-in with DNA polymerase. The DNA was then partially digested with *Xmn*I and ligated intramolecularly. The resulting transposon was missing the left Tn*3* end and polylinker, a small portion of the *URA3* gene and the 3′ *amp*^{R} sequences. The extent of the deletion (*left Tn3 end* 3′ *amp*) is shown in Figure 5

[a] In the plasmid constructions described above, restriction sites for enzymes with 5′ cohesive ends were sometimes removed by "filling-in" the recessed 3′ ends with DNA polymerase (SYMINGTON and PETES 1988). This protocol often creates a site for a different restriction enzyme. For example, filling-in a *Bst*EII site creates a *Mae*II site. This situation is indicated by the designation *Bst*EII$^-$(*Mae*II$^+$).

TABLE 2

Yeast strains

Name	Description, derivation, reference
Haploid parental strains[a]	
LS18	*α trp1-1 arg4-17 tyr7-1 ade6 URA3;* derived from XJ24-24a (SYMINGTON and PETES 1988); *URA3* insertion present in *LEU2-CEN3* interval
LS25-70d	*a leu2-Bst ade6 ura3 can1;* contains restriction site changes introduced into the G *Bam*HI fragment (SYMINGTON and PETES 1988)
AS4	Derivative of LS18 that lacks *URA3* insertion in *LEU2-CEN3* interval, constructed by transformation with pLS47, followed by 5-FOA selection for Ura$^-$ phenotype (BOEKE *et al.* 1984); this strain has all restriction site fill-ins in the C and D *Bam*HI fragments except the *Eco*RI site adjacent to the centromere
AS8	The strain LS25-70d has a mutant *ura3* gene inserted in chromosome *III* (SYMINGTON and PETES 1988); this gene was removed by two consecutive transplacements, the first with pLS36 and the second with pLS32. The resulting strain lacked the mutant *ura3* insertion and retained the G4B restriction site changes
AS13	*CAN1* derivative of AS8; the two-step transplacement procedure (SCHERER and DAVIS 1979) was used with the plasmid pSH2::URA3 to replace the *can1* allele with a *CAN1* allele
AS14	AS4 derivative constructed by two-step transplacement with pLS55; contains *Cla*I$^-$(*Nru*I$^+$) alteration near the centromere (Figure 1)
AS20	AS13 derivative constructed by two-step transplacement with pLS57; contains *Eco*RI$^-$(*Xmn*I$^+$) change near *CEN3* (Figure 1)
AS38	AS20 derivative constructed by two-step transplacement with pLS65; contains *Sal*I$^-$(*Pvu*I$^+$) alteration in *HIS4* to generate *his4-Sal* mutation
Control diploid strains lacking mini-Tn*3* insertions	
LS42 LS45 LS47	Related diploid strains (SYMINGTON and PETES 1988) with heterozygous restriction site markers between *LEU2* and *CEN3* (Figure 1). The strains were derived from a cross of LS18 to LS25-70d and have the genotype; a/*α TRP1/ trp1-1 LEU2/leu2-Bst ARG4/arg4-17 TYR7/tyr7-1 ura3-52/ura3 ade6/ade6*. LS42 is heterozygous for a mutant insertion of *ura3* in the G4B *Bam*HI fragment. LS45 is deleted for this insertion. LS47 is isogenic with LS45, except for the addition of the heterozygous *Cla*I marker near *CEN3*
AS17	AS13 × AS14; this diploid has the same genotype as LS47, except that it is homozygous for *CAN1*
AS29	AS14 × AS20; this diploid has the same genotype as AS17, except for an additional heterozygous marker (*Eco*RI$^-$) near the centromere
AS50	AS14 × AS38; this diploid has the same genotype as AS29, except for the addition of a heterozygous *his4-Sal* mutation
Haploid strains with mini-Tn*3* insertions	
AS21	AS20 with single-step transplacement (ROTHSTEIN 1983) of pAS55 into chromosome *III* (site 1 insertion; Figure 1)
AS22	AS20 with single-step transplacement of pAS56 (site 2 insertion)
AS23	AS20 with single-step transplacement of pAS57 (site 3 insertion)
AS28	AS14 with single-step transplacement of pAS55 (site 1 insertion)
AS35	AS14 with single-step transplacement of pAS56 (site 2 insertion)
AS27	AS14 with single-step transplacement of pAS57 (site 3 insertion)
AS39	AS38 with single-step transplacement of pAS56 (site 2 insertion); identical to AS22 except for additional mutation at the *HIS4* locus
AS76	AS4 with single-step transplacement of pAS55 (site 1 insertion)
AS77	AS8 with single-step transplacement of pAS55 (site 1 insertion)
Diploid strains with heterozygous insertions of mini-Tn*3*	
AS3	Derived from LS45 by one-step transplacement with pAS55 into the copy of chromosome *III* that contains the mutant *leu2* allele; heterozygous for insertion at site 1
AS6	Derived from LS47 by one-step transplacement with pAS55 transplaced into the copy of chromosome *III* that contains the mutant *leu2* allele; heterozygous for insertion at site 1
AS30	AS14 × AS21 (heterozygous for insertion at site 1)
AS31	AS14 × AS22 (heterozygous for insertion at site 2)
AS32	AS14 × AS23 (heterozygous for insertion at site 3)
Diploid strains with homozygous insertions of mini-Tn*3*	
AS9	AS76 × AS77 (homozygous for insertions at site 1)
AS34	AS21 × AS28 (homozygous for insertions at site 1); identical with AS9, except for additional restriction site markers near *CEN3* and homozygous *CAN1* alleles
AS36	AS35 × AS22 (homozygous for insertions at site 2)
AS40	AS35 × AS39 (homozygous for insertions at site 2); identical to AS36, except for heterozygous *his4-Sal* mutation
AS37	AS23 × AS27 (homozygous for insertions at site 3)

Name	Description, derivation, reference
Haploid strains containing deletion derivatives of mini-Tn*3*[b]	
AS42	Derivative of AS39 resulting from transplacement with pAS26; Ura$^-$ transformants were selected using 5-FOA to select for loss of the *URA3* gene from the insertion (*URA3* deletion)
AS44	Derivative of AS14 resulting from transplacement with pAS31; this train has a mini-Tn*3* element that is missing the *amp*^{R} gene (*amp* deletion)
AS46	Derivative of AS38 resulting from transplacement with pAS31; contains *amp* deletion
AS55	Derivative of AS38 resulting from transplacement with pAS101; has mini-Tn*3* insertion with a deletion of the 3′ portion of the *amp*^{R} gene (3′*amp* deletion)
AS59	Derivative of AS14 resulting from transplacement with pAS101 (3′ *amp* deletion)
AS62	Derivative of AS38 resulting from transplacement with pAS103; has mini-Tn*3* insertion with a deletion of both *loxP* and *amp*^{R} sequences (*loxP amp* deletion)
AS63	Derivative of AS14 resulting from transplacement with pAS103 (*loxP amp* deletion)
AS69	Derivative of AS38 resulting from transplacement with pAS106; has mini-Tn*3* insertion with deletions of 3′ end of element *and* 3′ end of *amp*^{R} (*left Tn3 end* 3′*amp* deletion)
AS70	Derivative of AS14 resulting from transplacement with pAS106 (*left Tn3 end* 3′*amp* deletion)
AS72	Derivative of AS55 resulting from transplacement with pAS105 (5-FOA selection); has mini-Tn*3* element with deletion of *URA3* and 3′ end of *amp*^{R} gene
Diploid strains homozygous for deletion derivatives of mini-Tn*3* insertion	
AS47	AS42 × AS35; mitotic conversion resulting in homozygous mini-Tn*3* (*URA3* deletion), insertion was selected on 5-FOA
AS48	AS44 × AS46 (homozygous for insertion with *amp* deletion)
AS58	AS59 × AS55 (homozygous for insertion with 3′*amp* deletion)
AS66	AS62 × AS63 (homozygous for insertion with *loxP amp* deletion)
AS71	AS69 × AS70 (homozygous for insertion with *left Tn3 end* 3′*amp* deletion)
AS75	AS72 × AS59, mitotic conversion resulting in homozygous mini-Tn*3* insertion (with *URA3* 3′*amp* deletion); selected on 5-FOA
Strains used for the analysis of gene conversion within the mini-Tn*3* insertion	
AS60	Derivative of AS39 generated by transplacement with pRB35; replacement of *URA3* gene in the mini-Tn*3* insertion with mutant *ura3* allele (*Nco*$^-$); insertion at site 2
AS61	AS60 × AS35 (heterozygous *URA3*/*ura3* in Tn*3* insertions at site 2)

[a] All strains used in these experiments are derived by transformation from the haploid strains LS18 and LS25-70d. All strains derived from LS18 contain the markers: *α trp1-1 arg4-17 tyr7-1* and *ade6*. All strains derived from LS25-70d contain the markers: *a leu2-Bst* and *ade6*. The markers introduced in the various strains include restriction site markers near *CEN3*, a *his4* mutation, a *CAN1* allele, and/or mini-Tn*3* insertions. A cross between two strain names indicates a mating of haploids to produce a diploid.

[b] All deletions involve the mini-Tn*3*::*URA3* insertion at site 2.

tures and treated with the enzymes *Bgl*II, *Mlu*I and *Xho*I. The fragments were separated on 0.8% agarose gels and analyzed as described above. At least four cultures that were sporulated independently were examined for each different diploid strain.

To look for double-strand breaks within the mini-Tn*3*::*URA3* insertion, we used a similar procedure. The diploid AS36 (homozygous for an insertion of mini-Tn*3*::*URA3* at site 2) was transferred to sporulation medium, and samples were taken every two hours. DNA was isolated from each sample, treated with *Xba*I and analyzed using the plasmid pAS35 as a hybridization probe. The *Xba*I fragment containing the insertion was about 5.1 kb in size; a double-strand break within the insert would generate two fragments approximately 2.5 kb in size. No such fragments were observed, although a faint band at a position of about 3 kb was observed in all samples (data not shown). This fragment was not meiosis-specific.

Statistical methods: The contingency chi-square test was used to compare any two sets of data that had more than five samples in each class and more than 25 samples total. Significance with one degree of freedom (at $P = 0.05$) is represented by a chi-square value of greater than 3.84. Any comparisons involving smaller numbers were done by the Fisher exact test or Monte Carlo simulations of the Fisher exact tests, using the StatXact program from Cytel Software Corp. Any P value less than 0.05 was considered to be significant. Other details concerning specific comparisons are given below.

Comparisons of crossovers in single intervals for different strains: One comparison was the frequency of simple crossovers in the recombinant interval containing the insertion compared to the frequency of simple crossovers in the control strain lacking the insertion; a simple crossover is signalled by tetratype segregation of a pair of adjacent restriction site markers, each marker showing 2:2 segregation. Since the insertion expands the size of the recombinant interval, it is necessary to normalize the data for the control strain in order to determine the expected frequency of recombination. For example, in the control strain, there were 44 simple crossovers in the 22-kb interval between *LEU2* and *CEN3* in 717 unselected tetrads; other *LEU2-CEN3* crossovers had a gene conversion event adjacent to the crossover. The rate of simple crossovers is, therefore, 2/kb/717 tetrads. Since the mini-Tn*3* insertion is 2.5 kb, one would expect about 5 crossovers/717 tetrads within the insertion, if the insertion has an average frequency of recombination for DNA in the 22-kb region. This value can be added to the number of observed crossovers in various control recombinant intervals in order to get an expectation for crossovers in strains with the homozygous insertion. For example, in the control strain, one crossover was observed

TABLE 3

Effect of insertions of mini-Tn*3* on *LEU2-CEN3* crossing over

Strain names	Insertion	Crossover ratio[a]	Percent crossovers[b]
Control strains (no insertions)			
LS42, LS45, LS47[c]	None	91/717	13
AS17, AS29	None	58/413	14
Strains with homozygous insertions of intact mini-Tn*3*::*URA3*			
AS9, AS34	Site 1 insertions	95/542	18
AS36, AS40	Site 2 insertions	125/318	39
AS37	Site 3 insertions	114/309	37
Strains with homozygous insertions of deletion derivatives of mini-Tn*3* at site 2			
AS47	*URA3* deletion	98/272	36
AS48	*amp* deletion	96/372	26
AS58	3′*amp* deletion	134/306	44
AS66	*loxP amp* deletion	64/320	20
AS71	*left Tn3 end* 3′*amp* deletion	125/314	40
AS75	*URA3* 3′*amp* deletion	128/298	43
Strains with heterozygous insertions of the intact mini-Tn*3* element			
AS3, AS6, AS30	Site 1 insertions	62/435	14
AS31	Site 2 insertions	34/239	14
AS32	Site 3 insertions	28/286	10

[a] The number of tetratype tetrads (*LEU2/TRP1*) divided by the total number of tetrads with four viable spores.
[b] The crossover ratio expressed as a percentage.
[c] Data from SYMINGTON and PETES (1988).

between the M and G4 restriction sites (Figure 2a). If a diploid was homozygous for the mini-Tn*3* insertion between M and G4, the expected number of crossovers would be 6/717 tetrads. When strains homozygous for the insertion at site 1 (AS9, AS34) were examined, 95 tetrads with a crossover between *LEU2* and *CEN3* were detected in 542 tetrads (Table 3). Forty-five of these tetrads were exained by Southern analysis to map the position of crossovers, and 14 simple crossovers were detected between M and G4 (Figure 2b). The effective sample size for the total tetrads for AS9 and AS34 is (45/95) × 542, or 257; no adjustment in the sample size for the control strains is necessary, since all 91 *LEU2-TRP1* tetratype asci were examined by Southern analysis (SYMINGTON AND PETES 1988). The comparison that was examined by contingency chi-square was: 6/717 (for the control) *vs.* 14/257 (for the homozygous insertion).

Similar calculations were done for strains homozygous for insertions at site 2 (20 tetrads examined by Southern analysis) and site 3 (31 tetrads examined by Southern analysis). For strains with heterozygous insertions, the following numbers of tetrads were examined by Southern analysis: 53 (total number analyzed for strains AS3, AS6, AS30), 30 (AS31), and 19 (AS32). The number of tetrads dissected for each strain and the number of tetrads with a crossover between *LEU2* and *CEN3* are shown in Table 3.

The data summarized in Figure 2 include mapping information from tetrads with single simple crossovers (class 1), as well as information from some of the tetrads with multiple recombination events (class 3). The data summarized in Figure 4 include mapping information from tetrads with single conversion-associated crossovers (class 2), as well as information from some Class 3 tetrads.

Comparison of distribution of crossovers in different strains: These comparisons were made by assigning the number of crossovers in each interval to separate classes. Any classes with no members in the control and in the experimental strain were deleted. This procedure produces a 2 × (number of classes) table that was then analyzed using the StatXact program. This program calculates the probability (*P*) that the distribution of events in the two classes is different. For strains with homozygous insertions, the expected number of crossovers (for the control strains) in the interval containing the insertion was adjusted for the size of the insertion. As described in the previous section, this adjustment was based on the average frequency of crossing over for DNA in the region analyzed. The size of the interval was not adjusted for heterozygous insertions.

Comparison of distribution of conversion tracts in different strains: These comparisons were similar to those described for the crossover distributions. Tracts were assigned to classes based on the sites that started and ended the tract. The distributions were compared by using the StatXact program. For homozygous insertions, any class in which the conversion event included sites that spanned the insertion were not used in the comparisons, since we assume that such events would be rarer for longer intervals. For heterozygous comparisons, such classes were included.

RESULTS

Experimental system: The pattern of meiotic recombination occurring in the 22-kb region between *LEU2* and centromere of chromosome *III* has been investigated previously (SYMINGTON and PETES 1988). The positions of crossovers and conversions in this region were determined using heterozygous markers caused by abolishing restriction sites from one of the two homologous chromosomes. The positions of these sites are indicated in Figure 1. In strains heterozygous at the *LEU2* locus and heterozygous for a mutation at *TRP1* (a tightly centromere-linked marker on chromosome *IV*), crossovers between *LEU2* and *CEN3* result in tetrads with tetratype segregation (1 Leu^+Trp^+:1 Leu^+Trp^-:1 Leu^-Trp^+:1 Leu^-Trp^- spores). DNA was isolated from spore cultures derived

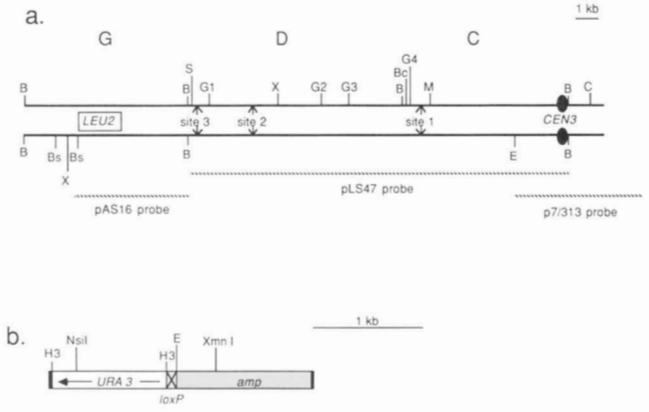

FIGURE 1.—Restriction maps of relevant DNA sequences. (a) Arrangement of restriction sites on chromosome III homologs between *CEN3* and *LEU2*. The heterozygous restriction sites used to map recombination events are marked (B = *Bam*HI, Bs = *Bst*EII, X = *Xho*I, S = *Spe*I, G = *Bgl*II, Bc = *Bcl*I, M = *Mlu*I, E = *Eco*RI, C = *Cla*I). Except for the *Bam*HI sites, this map (as well as those in Figures 2, 4 and 6) shows only those restriction sites that were heterozygous; numerous other sites exist for these enzymes that are not shown. The letters G, D and C correspond to the *Bam*HI fragments that were used to construct strains with heterozygous markers; the procedures used to delete restriction sites and to construct the heterozygous diploid yeast strains are described in Tables 1, 2 and SYMINGTON and PETES (1988). We also show the positions at which the mini-Tn*3*::*URA3* elements were inserted (one insertion per strain). Below the map are indicated the location of hybridization probes used to examine the segregation of the heterozygous sites. (b) Arrangement of DNA sequences in the mini-Tn*3*::*URA3* element. The element is flanked by 38 bp repeats derived from Tn*3*. The transcriptional orientation of the *URA3* gene is indicated by the arrow. The β-lactamase gene is transcribed in *E. coli* in the rightward direction with respect to the map. H3 = *Hind*III.

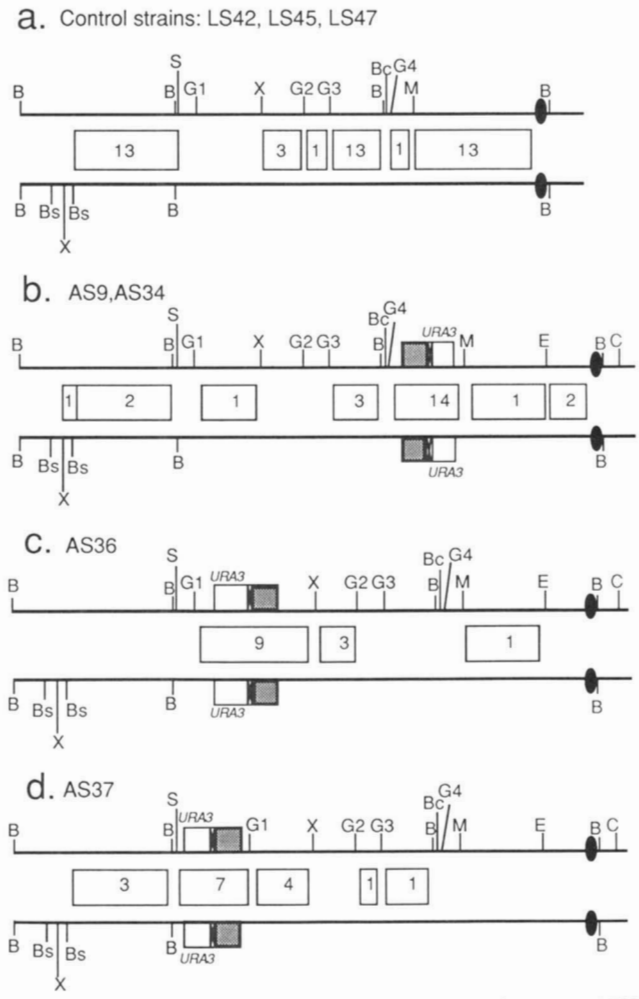

FIGURE 2.—Distributions of simple crossovers between *CEN3* and *LEU2* in strains homozygous for the insertion of mini-Tn*3* and in strains lacking the insertion. The positions of these crossovers were mapped as described in MATERIALS AND METHODS; the numbers of tetrads analyzed by Southern analysis for each strain are also described in this section. All heterozygous restriction sites were analyzed. Simple crossovers represent tetrads in which two heterozygous sites (each segregating 2:2) are reciprocally recombined. The boxed numbers in the figure represent the number of tetrads with a simple crossover in the recombinant interval. Restriction sites are abbreviated as in Figure 1. (a) Pattern of crossovers in the control strains lacking the mini-Tn*3* element (data from SYMINGTON and PETES 1988). (b–d) Pattern of crossovers in strains homozygous for the mini-Tn*3* insertion at site 1 (b), site 2 (c) and site 3 (d). The position of the insertion is indicated by a rectangle, with the *URA3* gene indicated by the white portion and the amp^R gene indicated by the gray portion.

from such tetrads and examined for segregation of the heterozygous restriction site markers by Southern analysis. The region between the restriction sites G3 and B (control strain, Figure 2a) was a hotspot for crossovers and the region between sites G1 and X was a coldspot. Subsequent studies indicated that the crossovers that occur between G3 and B are initiated between the B and M sites (L. SYMINGTON and T. PETES, unpublished data).

We have examined the effects of inserting a 2.5-kb sequence derived from the transposable element Tn*3* on the pattern of meiotic exchange in the *LEU2-CEN3* interval. This element (mini-Tn*3*::*URA3*) contains the wild-type *URA3* allele, about 100 bp of polylinker, the beta-lactamase gene (amp^R) derived from pBR322, the *loxP* site derived from bacteriophage P1, and 38-bp repeats from Tn*3* (SEIFERT *et al.* 1986; see Figure 1b). When transposase is provided in *trans*, this element is capable of transposition in *E. coli*. Using the protocol developed by SEIFERT *et al.* (1986), we selected recombinant plasmids (containing yeast DNA sequences derived from the *LEU2-CEN3* interval) that had insertions of the mini-Tn*3* element (Table 1). Plasmids with three different sites of insertion were used in subsequent yeast strain constructions; the positions of the insertions are indicated in Figure 1a. These plasmids were transformed into haploid yeast strains using either one- or two-step transplacement procedures (see MATERIALS AND METHODS and Table 2). The net results of these manipulations are strains in which the normal chromosomal sequences have been replaced by those that contain the insertion. The resulting haploid strains were mated to yield diploid strains that were either heterozygous or homozygous

for insertions of mini-Tn*3*::*URA3* at sites 1, 2 or 3 (Figure 1). In addition, the strains were heterozygous for the restriction site markers used in our previous study. Except for changes introduced by transformation, all diploid strains used in these studies are isogenic.

Homozygous insertions of mini-Tn*3* stimulate crossing over: As described above, the frequency of crossing over between *LEU2* and *CEN3* is measured by the frequency of tetratype asci for the *LEU2* and *TRP1* markers. As shown in Table 3, in strains with no insertions (LS42, 45 and 47), about 13% of unselected tetrads had a crossover in the *LEU2-CEN3* interval. The same level of crossovers was observed in AS17 and AS29; these strains are isogenic with the LS42, 45 and 47 strains but were constructed by a different procedure (Table 2). The level of recombination observed in all control strains is an average amount of recombination for a 22-kb interval (STRATHERN *et al.* 1979). Strains with homozygous mini-Tn*3* insertions at any of the three sites have significantly elevated levels of crossing over between *LEU2* and *CEN3*; the respective contingency chi-square values for comparisons of strains with site 1, 2 and 3 insertions with the control strains were: 5.4 (*P* less than 0.05), 93 (*P* less than 0.001), and 77 (*P* less than 0.001). Strains with insertions at sites 2 (AS36, AS40) and 3 (AS37) have 3-fold elevated levels of crossing over between *LEU2* and *CEN3*, whereas the level of stimulation observed in strains with site 1 insertions (AS9, AS34) was about 50% (Table 3); although the stimulation of crossing over is observed for all homozygous strains, the stimulation observed for the strains AS9 and AS34 is significantly less than for the other strains with homozygous insertions (chi-square value of 49 when compared to AS36 and AS40; chi-square value of 39 when compared to AS37). One explanation for this result is that insertion of mini-Tn*3* into site 1 disrupts the recombination events that normally initiate in this region. As described above, in strains without the insertion, the region between the *Bam*HI and *Mlu*I sites acts as a site for the initiation of recombination. Thus, recombination between *LEU2* and *CEN3* in strains with the site 1 insertion may be stimulated by only one hotspot (provided by the insertion) whereas, in strains with the other insertions, recombination may be stimulated by two hotspots, the endogenous hotspot between the B and M sites (see above) and the hotspot provided by the insertion.

We next investigated whether the mini-Tn*3* insertion stimulated meiotic crossing over for the entire *LEU2-CEN3* region or stimulated recombination locally. Using the heterozygous restriction site markers present in the strains that were homozygous for the mini-Tn*3* insertions, we mapped the position of the crossovers by Southern analysis (BORTS and HABER 1987; SYMINGTON and PETES 1988). As shown in Figure 2, most of the crossovers occur in the interval containing the insertion; a statistical analysis (described in MATERIALS AND METHODS) indicates that this local stimulation is significant [chi-square values of 17.8 (AS9, AS34), 68.5 (AS36), and 24.7 (AS37); *P* less than 0.001] for all three insertions. This result indicates that the crossovers stimulated by the insertion occur within or near the insertion.

For insertions at sites 2 and 3 (but not site 1), crossing-over is significantly elevated in one chromosomal interval bordering the insertion (the X-G2 interval for site 2 and the G1-X interval for site 3). This result suggests the possibility that some of the events initiated within the insertion may be resolved elsewhere. A similar suggestion may explain the observation that deletion of the B-M region significantly reduces the level of crossing over in the G3-B and M-*CEN3* intervals (L. SYMINGTON and T. PETES, unpublished data).

The stimulation of crossing over by the insertion can also be demonstrated by physical analysis of DNA isolated from the spores. As shown in Figure 3, crossovers that occur between heterozygous restriction sites produce DNA fragments that are altered in mobility relative to the parental fragments. The intensity of the recombinant bands is much greater in the strain AS36 (homozygous for the insertion at site 2) than in the strain AS50 which lacks the insertion.

Double-strand breaks in meiotic DNA of *S. cerevisiae* have been detected at the *ARG4* locus (SUN, TRECO and SZOSTAK 1989) and near the *LEU2* gene in a strain containing a duplication of these sequences (CAO, ALANI and KLECKNER 1990). Consequently, we isolated DNA from AS36 during sporulation and examined *Xba*I-treated DNA samples by Southern analysis in order to detect double-strand breaks in the insertion. *Xba*I digestion results in a fragment of about 5.1 kb in AS36; double-strand breaks within the insertion would lead to two fragments of about 2.5 kb. Although we estimate that we could detect such fragments if they occurred at a 1% level, none were observed. A faint band was observed at a position of about 3 kb; however, this band was present in all DNA samples (including the premeiotic sample). These results indicate that either double-strand breaks within the insertion are not responsible for initiating crossing over or that meiosis in this strain is not sufficiently synchronous to allow detection of the transient breaks.

Homozygous insertions of mini-Tn*3* stimulate gene conversion: In our previous study of LS42, LS45 and LS47 (SYMINGTON and PETES 1988), 53% of the crossover tetrads had simple crossovers (class 1), 26% had conversion events adjacent to the position of the crossover (class 2), and 21% had complex recombination events (more than one crossover, more than one conversion event, etc.; class 3). The percent-

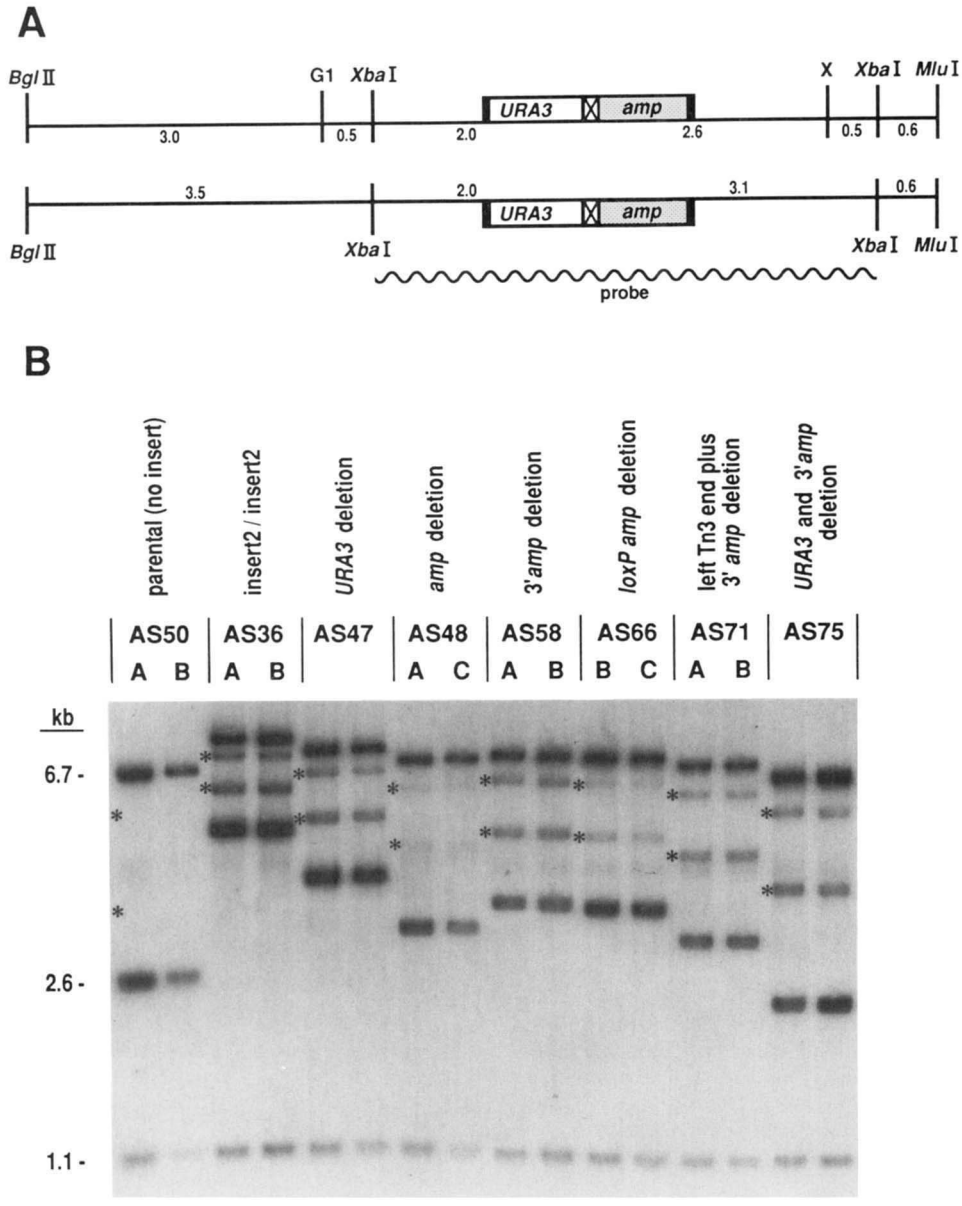

FIGURE 3.—Physical detection of recombination by Southern analysis. Diploid yeast strains lacking a mini-Tn*3* insertion (lanes 1 and 2), homozygous for the intact mini-Tn*3* insertion at site 2 (lanes 3 and 4), or homozygous for various deletion derivatives of the mini-Tn*3* insertion at site 2 (lanes 5–16) were sporulated and DNA was isolated after three days of sporulation. DNA was triply digested with the restriction enzymes *Bgl*II, *Mlu*I, and *Xho*I and the resulting fragments were separated by gel electrophoresis. After transfer of DNA to a filter, the fragments were hybridized to ^{32}P-labeled pAS35 plasmid sequences (Table 1). (a) Location of the *Bgl*II, *Mlu*I, and *Xho*I sites in the two parental chromosomes for a strain homozygous for the intact mini-Tn*3* insertion. The vertical lines labeled G1 and X correspond to the heterozygous *Bgl*II and *Xho*I sites shown in Figure 1. The homozygous *Bgl*II, *Xba*I, *Nde*I, and *Mlu*I sites shown in this figure are not shown in Figure 1. The numbers indicate the size of each fragment in kb. The region of homology to pAS35 is shown. The expected sizes of restriction fragments (in the absence of recombination) for the *Bgl*II-*Mlu*I-*Xho*I-treated DNA are 5.1 kb and 1.1 kb for one parent, and 9.2 kb for the other. Recombination in the interval spanned by the heterozygous G1 and X sites would result in fragments of 6.2 kb and 8.1 kb. The expected fragment sizes in the diploid strain without the insertion are 2.6 kb and 0.5 kb for one parent, and 6.7 kb for the other. (b) Southern analysis of various sporulated diploid strains. As described above, the isolated DNA was treated with *Bgl*II, *Mlu*I and *Xho*I for analysis. For most of the strains, two independently constructed diploids were analyzed (indicated by capital letters above the lanes). For those strains with only one isolate of the diploid, two independently sporulated cultures were examined. Asterisks mark the expected positions of recombinant fragments. No recombinant fragments can be detected in this exposure in the control strain (AS50) lacking the insert.

ages of class 1:class 2:class 3 tetrads in the experimental strains were: 31:27:42 (strains homozygous for site 1 insertions), 50:40:10 (strains homozygous for site 2 insertions), and 42:52:6 (strains homozygous for site 3 insertions). The classes of conversion events detected in these strains are shown in Figure 4. Many of these events involve restriction sites that border the insertion, as expected if the insertions initiate both conversion events and crossovers. The distribution of conversion tracts in all experimental strains is significantly altered compared to the distribution observed for the control strain; in comparing the distribution

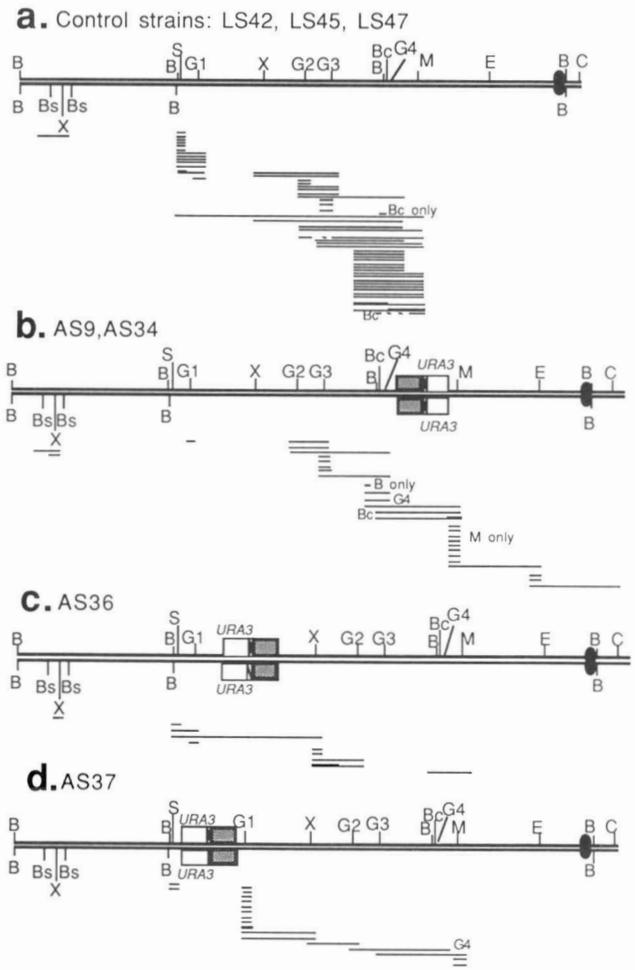

FIGURE 4.—Distribution of conversion tract lengths in diploid strains with homozygous insertions of the mini-Tn*3*::*URA3* element. As described in the legend to Figure 2, we mapped the segregation of heterozygous restriction site markers in tetrads with a crossover between *LEU2* and *CEN3*; the number of tetrads examined by Southern analysis for each strain is given in the MATERIALS AND METHODS. In about one-third of such tetrads, the crossover was associated with gene conversion of one or more adjacent markers. The minimal lengths of these conversion events are indicated by the horizontal lines in the figure. Each line represents a conversion tract in a different tetrad. In general, these tracts were continuous, although some tracts had regions of 2:2 (indicated by dotted lines) or 4:0 or 0:4 (indicated by thick lines) segregation within a 3:1 or 1:3 tract. Since the B, Bc and G4 sites are tightly clustered, for tracts that end or begin in this region of the chromosome, the designation of the last site included in the tract is given. (a) Conversion tract patterns in strains lacking the mini-Tn*3* insertion (data from SYMINGTON and PETES 1988). (b–d) Conversion tract patterns in strains homozygous for insertions of the mini-Tn*3* element at site 1 (b), site 2 (c), or site 3 (d).

of conversion events for strains homozygous for the insertions with the control strains (Fisher exact test; see MATERIALS AND METHODS),we obtained the following *P* values: site 1 strains ($P = 0.002$), site 2 strains ($P = 0.046$), and site 3 strains ($P = 0.001$). If the conversion events occurring at sites flanking the insertion are examined, we find that the insertions at sites 2 and 3 have significantly elevated levels of conversion of the flanking sites (*P* values of 0.001 and 0.024, respectively), whereas the insertion at site 1 does not result in a significant elevation (*P* value of 0.19). The lack of an effect for the site 1 insertion is not entirely unexpected, since the region containing site 1 has a high level of gene conversion, even in the absence of the insertion (SYMINGTON and PETES 1988).

We also examined the rate of gene conversion *within* the mini-Tn*3* insertion. We considered a diploid strain AS61 that was homozygous for the mini-Tn*3* insertion; in one copy of the insertion, the *URA3* gene was mutated by filling-in an *Nco*I restriction site within the coding sequence; this process converts the *Nco*I site to an *Nsi*I site. In this strain, we found that 50 of 245 unselected tetrads (20%) had a conversion event involving *URA3*. This rate of conversion is much higher than that observed for most yeast genes, consistent with the possibility that the mini-Tn*3* element stimulates both crossing over and gene conversion. In AS61, there were 12 $3^+:1^-$ and 38 $1^+:3^-$ convertants. Since the AS61 strain has mutant copies of the *ura3* gene on chromosome *V* (its normal location) as well as on chromosome III, we isolated and examined DNA from spore cultures derived from 12 $1^+:3^-$ tetrads in order to show that the conversion events were allelic rather than ectopic. All of the Ura^- spores had a *ura3* gene with the *Nsi*I restriction site, as expected if the conversion events involve allelic interactions between the mini-Tn*3* elements.

The 5′ end of the β-lactamase gene within the element is required for full hotspot activity: As shown in Figure 1, the 2.5-kb mini-Tn*3*::*URA3* element contains a number of DNA sequences derived from different sources. To find out whether the hotspot activity was localized in one portion of the element, we analyzed deletion derivatives of the element inserted at site 2. The extent of these deletions is shown in Figure 5. The effects of each deletion were examined by constructing diploid strains homozygous for these derivatives. Two types of analysis were done on each strain: determination of the frequency of crossing over between *LEU2* and *CEN3*, and a physical analysis of the proportion of recombinant band presented in meiotic products.

Data from the segregation analysis are shown in Table 3. Several conclusions can be drawn from these data. First, deletions that remove the 5′ end of the amp^R gene significantly (contingency chi-square values of 14 (*P* less than 0.001) for AS48 and 28 (*P* less than 0.001) for AS66) reduce the activity of the hotspot relative to that observed in strains with the intact element (AS36 and AS40). Second, many of the deletions (for example, deletion of the *URA3* gene) have no effect on hotspot activity. Third, no single deletion completely inactivates the hotspot. This result sug-

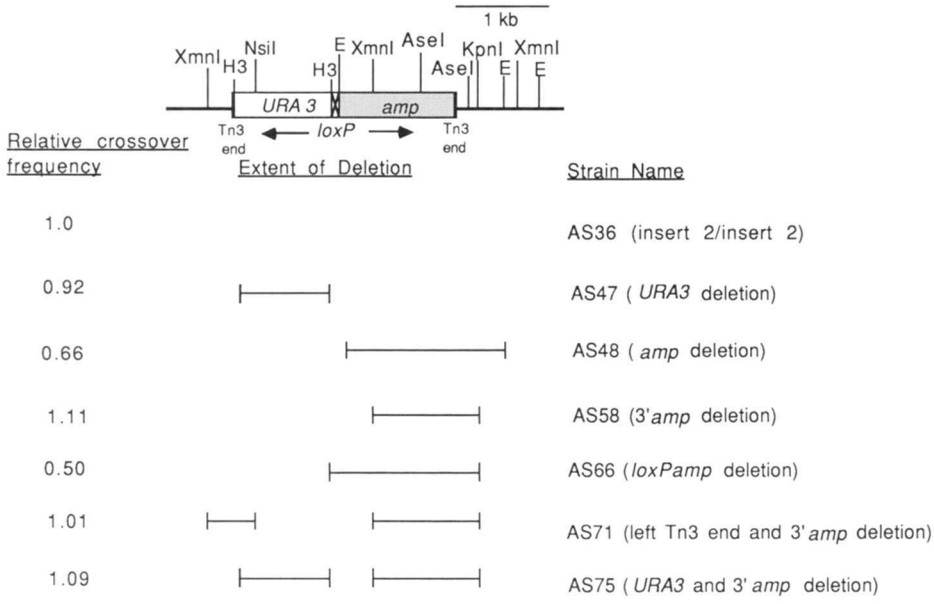

FIGURE 5.—Deletion derivatives of the mini-Tn*3*::*URA3* element located at site 2. A partial restriction map of the element and flanking yeast DNA sequences at site 2 is shown. Arrows indicate the direction of transcription. Deletions of this element were constructed (as described in Table 1) and the extent of this deletion is indicated by the horizontal lines. Diploid yeast strains homozygous for these deleted elements were constructed (as described in Table 2) and the frequency of *LEU2-CEN3* crossing over was monitored. The crossover ratio represents the frequency of *LEU2-CEN3* crossover tetrads in the strain containing the deleted element divided by the frequency fo *LEU2-CEN3* crossover tetrads in the strain (AS36) containing the intact element.

gests that there may be more than one sequence within the insert that has hotspot activity.

The conclusions from the segregation analysis are supported by the physical studies (Figure 3). Both deletions that remove the 5′ end of the amp^R gene decrease the amount of recombinant product, whereas other deletions have no obvious effect. It is striking that a 400-bp sequence, consisting of the 5′ end of the amp^R gene, the *loxP* site, a polylinker, and a 38-bp Tn*3* terminal repeat, has about the same recombinogenic activity as the intact element (compare recombinant bands in strain AS75 with those in the control strain AS50). This 400 bp sequence increases *LEU2-CEN3* recombination by about 15cM (Table 3). Since the average amount of recombination in yeast is about 0.37 cM/kb, this hotspot is about 100-fold "hotter" than an average yeast sequence.

We conclude that the 5′ end of amp^R gene is responsible for at least half of the hotspot activity. There are, however, other sequences within the mini-Tn*3* insertion that are capable of stimulating crossing over.

Heterozygous insertions of mini-Tn*3* do not stimulate crossing over or gene conversion: In contrast to the effects observed with the homozygous insertions, heterozygous insertions of the intact element failed to stimulate either conversion or crossing over. As shown in Table 3, no significant increase was observed in crossovers between *LEU2* and *CEN3* in strains heterozygous for elements at sites 1, 2 or 3 (contingency chi-square values of 0.44, 0.25 and 1.4, respectively). When the positions of the crossovers were mapped (Figure 6), no statistically significant clustering of crossovers near the position of the insertion was detected for strains with heterozygous elements at sites 1, 2 or 3 (contingency chi-square values of 1.00, 0.06 and 0.10, respectively). Although the

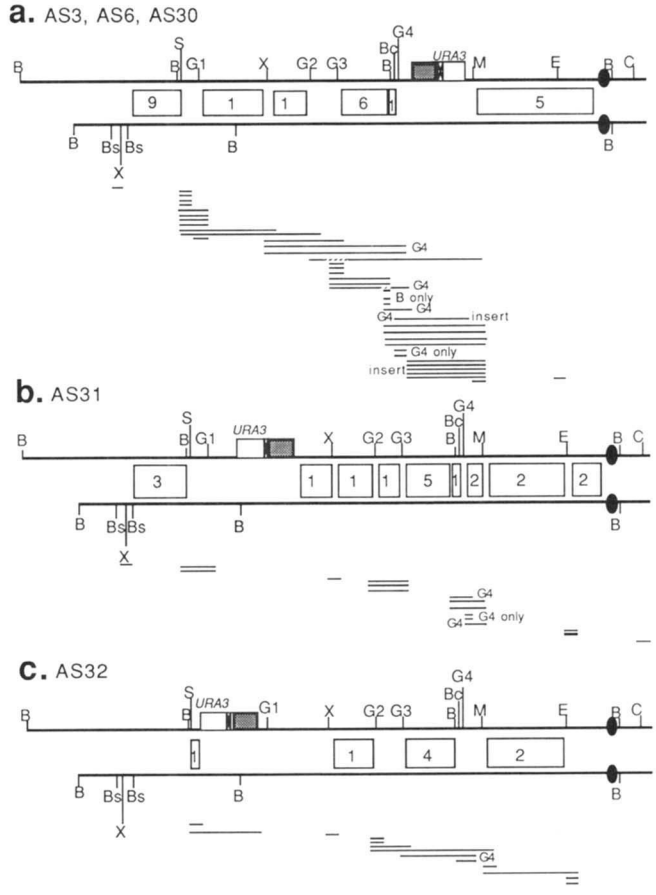

FIGURE 6.—Distributions of crossovers and conversion tracts in yeast strains heterozygous for the mini-Tn*3* element. Diploid strains were constructed that were heterozygous for insertions at site 1 (a), site 2 (b), or site 3 (c). These strains were analyzed by the procedures described in the legends to Figures 2 and 4.

distributions of conversion tracts in strains heterozygous for insertions in sites 2 and 3 were not different from the control strain (*P* values of 0.139 and 0.389,

respectively), this distribution of tracts in strains heterozygous for insertions at site 1 was significantly different (P value of 0.023). In addition, the heterozygous insertion at site 1 has a higher level of conversion (21/435 tetrads segregated either 3:1 or 1:3 for the insertion) compared to strains heterozygous for the insertion sites 2 (1/239 conversion rate) or 3 (3/375 conversion rate). Both of these results suggest that the insertion in site 1 affected (and is affected by) the endogenous recombination hotspot that is known to exist at this position.

DISCUSSION

We have shown that the mini-Tn*3* transposon derived from a bacterial plasmid stimulates meiotic crossing over and gene conversion in *S. cerevisiae*. The degree of stimulation is somewhat dependent on the context of the element in the chromsome. Most of the recombination events induced by the element occur within or nearby the element. The stimulation is observed only when the insertion is homozygous, unlike observations made with the M26 hotspot in *S. pombe* (GUTZ 1971). The 5′ end of the amp^R gene is essential for full hotspot activity.

In two other studies, meiotic recombination has been examined in strains with the amp^R gene present as a homozygous insertion. BORTS and HABER (1987) constructed diploid strains in which the plasmid pBR322 (which contains the same amp^R gene as the mini-Tn*3* insertion) and *URA3* were inserted between duplicated copies of the MAT loci. They found that these sequences had approximately 4-fold more crossing over than an average chromosomal region. No deletion analysis was done to determine where the hotspot activity resided. R. MALONE (personal communication) used the mini-Tn*3*::*URA3* element to separate a putative endogenous hotspot for meiotic gene conversion (located 3′ to *HIS2*) from the *HIS2* structural gene. These insertions reduced the level of gene conversion at *HIS2*. Since we find that the mini-Tn*3* element stimulates meiotic gene conversion, this observation is puzzling. It may reflect a complex interaction between the endogenous hotspot at *HIS2* and the mini-Tn*3* element, similar to the effects that we observed with insertions in site 1.

In two popular models, recombination is initiated either by a single-strand nick that is expanded into a single-strand gap (MESELSON and RADDING 1975; RADDING 1982) or by a double-strand break in the DNA (SZOSTAK *et al.* 1983). In the context of these models, there are two likely explanations of the hotspot activity of the mini-Tn*3* insertion. One possibility is that the enzymes that initiate recombination preferentially recognize certain DNA sequences and these sequences are located within the insertion. Alternatively, the enzymes that initiate recombination may nonspecifically cleave DNA, but the accessibility of DNA to these enzymes varies from one chromosomal region to another. For example, the mini-Tn*3* hotspot may be less covered in nucleosomes or other chromosomal proteins than an average chromosomal region. In distinguishing between these possibilities, it would be useful to examine the meiotic chromatin structure of hotspots, in order to determine whether these regions tend to be hypersensitive to nucleases.

As mentioned previously, double-strand breaks have been detected at both the *ARG4* (SUN, TRECO and SZOSTAK *et al.*, 1989) and ectopic *LEU2* loci (CAO, ALANI and KLECKNER 1990) in yeast. Although we have not detected such breaks in the mini-Tn*3* insertion, the strain used in our study undergoes meiosis more slowly (and, perhaps, more asynchronously) than the strain used in the other studies. We have not yet examined the mini-Tn*3* DNA for meiosis-specific single-stranded nicks.

The observation that the mini-Tn*3* insertion does not function as a hotspot as a heterozygous insertion can be explained by both recombination models. In both models, after the initial lesion is made, the resulting chromosomal ends or gaps interact with the homologous chromosome. This interaction involves heteroduplex formation and, therefore, requires sequence homology. Thus, if the initiating lesion occurs on the mini-Tn*3* element and the element is heterozygous, there is no homology with the homologous chromosome lacking the insertion until the gap (single- or double-stranded) is expanded beyond the boundaries of the element. Since the element is 2.5 kb in size, this nuclease-mediated expansion may be rare. Since the M26 hotspot represents a single bp change, the chromosome containing the hotspot shares almost complete homology with the homolog lacking the hotspot. The observation that the mini-Tn*3* hotspot does not function when heterozygous, therefore, is not surprising.

In conclusion, we showed that the 5′ end of the beta-lactamase gene greatly stimulates both meiotic gene conversion and crossing over. A DNA sequence of about 400 bp is sufficient for this effect. The small size of this hotspot should allow a more detailed analysis of the specific DNA sequences necessary for hotspot activity, as well as a physical analysis of meiosis-specific DNA lesions within the hotspot.

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